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Genetic Epidemiology of HbS in Oman: Multicentric Origin for the β^S Gene

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On the basis of a sample of 117 chromosomes, we have demonstrated the multicentric origin of the sickle mutation in Northern Oman. Three major haplotypes coexist: 52.1% Benin (typical and atypicals), 26.7% Arab-India, and 21.4% Bantu. These haplotypes are not autochthonous to Oman but originated elsewhere and arrived in Oman by gene flow. The distribution of haplotypes is in excellent agreement with the historical record, which establishes clear ancient contacts between Oman and sub-Sahara west Africa and explains the presence of the Benin haplotype; contacts with Iraq, Iran, present-day Pakistan, and India explain the presence of the Arab-India haplotype. More recent contacts with East Africa (Zanzibar/Mombasa) explain the presence of the Bantu haplotype. The pattern of the Arab-India haplotype in the populations of the Arabian peninsula reinforces the hypothesis that this particular mutation originated in the Harappa culture or in a nearby population and in addition reveals that the Sassanian Empire might have been the vehicle by which this Indo-European sickle mutation migrated (gene flow) to the present-day Arabian peninsula, including Oman. Am. J. Hematol. 64:39–46, 2000.

Key words: HbS; Oman; β^S gene

INTRODUCTION

The population of the Arabian Peninsula is characterized by a high incidence of hemoglobinopathies, including α- and β-thalassemia, HbS and HbC [1].

In the Arabian Peninsula the β^S gene is quite frequent. In Saudi Arabia the gene frequency varies from 0 (in the no malaric northern provinces) to 0.145 in the Eastern provinces (AS = 21.3%) [2]. Of particular interest are the Western provinces of Saudi Arabia in which the β^S is linked to Benin haplotype of African origin, while in the Eastern provinces it is linked to the Arab-India haplotype of Indo-European origin [3,4]. The clinical picture of sickle cell anemia linked to Benin and Bantu haplotype is more severe than that of the disease linked to the Arab-India haplotype [5–8]. In Bahrain [9] and Kuwait [10], the Arab-India haplotype predominates among carriers of the sickle gene.

The incidence of sickle trait (AS) in the rest of the Arabian Peninsula is less known. In a study done in pregnant women, consulting in a hospital in the United Arab Emirates (UAE) [11], incidence is low among Yemenites (0.95%), higher in UAE nationals (1.9%), and much higher in Omanis (3.8%). In a more precise Omani study, the AS frequency was 6.2% [12]. The gene frequency of α-thalassemia among Omanis in this study was very high (0.6), resulting in only about 11% of the population being αα/αα, 44% −α/αα, and 45% −α/−α [12]. Finally, according to a very recent report [13], using hospital-based data, the frequency of AS was 10%, but this could be a misestimation due to bias of ascertainment (hospitalized subjects).

The incidence of sickle cell anemia (SS) patients should be higher than expected due to consanguinity, estimated to be 56% (24% first cousins) [13]. Calculating the attributable fraction, Rajab and Patton conclude that consanguinity accounts for 17% of the cases of SS. Also the presence of small endogamic villages tends to increase or decrease the frequency of heterozygotes and/or homozygotes in some sites by the founders effect and/or genetic drift. An analysis of haplotype of a small number of chromosomes by these authors revealed the linkage of the β^S gene only to the Benin and Bantu haplotype [13].

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The work reported here involves the use of β-gene cluster haplotypes as means of identifying uni- or multicentric origin of a mutation, genetic flow, and their influence on hematological parameters [14]. The β-gene cluster haplotypes are a series polymorphic sites definable by endonuclease enzymes that serves as markers for a given chromosome. Only one of the polymorphic sites (*Xmn*I 5′ to $^{G}\gamma$; which determines the down-regulation of $^{G}\gamma$ after birth [15]) has a functional effect [16]. The rest, up to now, are considered markers of the chromosome.

The β-gene cluster haplotypes have been particularly useful in genetic epidemiology, making it possible to determine the emergence and expansion of the β^S gene in Africa, independently at least four times, and to define the geographical areas affected by each one of these mutational events: the Senegal haplotype (in Atlantic West Africa), the Benin haplotype (in central West Africa), and the Bantu haplotype in central, east, and southern Africa [13]. In addition, the Eton people of The Cameroons, living south of Younde, have a private (not found in any other ethnic group) fourth haplotype linked to the β^S gene, the Cameroon haplotype [17].

In addition to the African-associated sickle gene, there is another β^S gene linked to a different haplotype, called Arab-India, that has been found among individuals living in the eastern oasis of Saudi Arabia and among the tribes of India [5]. This Indo-European mutation probably arose 4,000–5,000 years ago, and the most likely place of origin is the Harappa culture of the Indus valley, since development of agriculture is indispensable for the significant expansion of the frequency of the gene. This advantage β^S renders the carrier partial protection from dying of malaria [18]. From phenotypes homozygous for the sickle gene, it has been possible to predict that the β^S gene would be found among other peoples of the Caucasus and contiguous areas (Azerbaijanis, Baluchistanis, Iranians, Iraqis, and Afghans). The very high HbF associated with sickle cell anemia in these populations, which is characteristic of the Arab-India haplotype, strongly suggests this linkage, but direct evidence is not available [19,20].

Little is known about the molecular characteristics of the HbS and HbC present in Oman. In the present study we have characterized a number of individuals carrying HbS, HbC, and HbSC in order to determine their haplotype linkage.

As a result of this study we have characterized the multicentric origin of HbS in Oman, related the genetic epidemiology to the historical record, and defined some hematological characteristics of the patients carrying different haplotype linkages.

MATERIALS AND METHODS

Blood samples from adult patients with sickle cell disease attending routine outpatient clinics were collected separately in NaEDT and CPD-A. NaEDTA samples were analyzed using cation-exchange high performance liquid chromatography (HPLC) using the Bio-Rad variant system [21,22]. The HbA;i2 was obtained by elution following Hb electrophoresis (Beckman Paragon electrophoresis system). DNA was extracted using standard protocols. Haplotyping of the β-gene cluster was carried out by PCR-based procedures as described elsewhere [23–25]. Ten sites were investigated: *Afl*III 5′ to ε, *Hin*cII 5′ to ε, *Xmn*I, 5′ to $^{G}\gamma$, *Hin*dIII within $^{G}\gamma$, and $^{A}\gamma$, *Taq*I 5′ to $^{A}\gamma$, *Hin*cII (two sites) within and 3′ to ψβ, *Rsa*I 5′ to β, and *Hin*fI 3′ to β. DGGE was used to determine the chromosomal framework [26]. When the set of polymorphic sites needed to ascertain the haplotype was not unequivocal, family studies were performed so to obtain a definitive assignment.

RESULTS

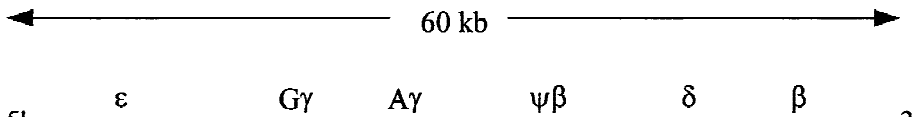
A total of 117 chromosomes linked to the β^S gene from the hospital of the Sultan Qaboos University were characterized as to their β-gene-like cluster haplotype. The findings are summarized in Table I. Typical Benin haplotype was found in 57 of the 117 chromosomes, and four were atypical Benin (haplotypes in which the 3′ region, encompassing the β globin gene and surrounding sequences, is typical Benin haplotype, but the 5′ region is different). Twenty-four chromosomes were linked to typical Bantu haplotype, with one atypical Bantu haplotype. Finally, 30 chromosomes exhibited typical Arab-India haplotype, with one atypical Arab-India haplotype. Hence of the chromosomes studied, 61 were Benin (typical and atypical) (52.1%), 31 were Arab-India (26.7%), and 24 were Bantu haplotypes (21.4%).

In Table II we depict 52 patients with SS genotypes and their HbF levels and range. Two aspects emerge from this picture: First, that the Arab-India haplotype in the homozygote form is associated with higher levels of HbF as compared to the Benin and Bantu haplotypes, and second, that the Arab-India haplotype in the heterozygous form, even with its own atypical form, has an average HbF level lower than homozygous cases of this haplotype ($N = 10$); however, the range establishes that some individuals with this genotype have high HbF comparable with that of the homozygous form.

Table III depicts the results on S/thalassemia and SC disease. We found examples of Benin, Bantu, and Arab-India haplotypes combined with β-thalassemia chromosomes, and the HbF level and range are very similar among them. The case of SC disease is a combination of a Bantu and a Benin haplotype.

Table IV depicts the haplotypes in 23 chromosomes of *normal* Omani chromosomes, in which one haplotype predominates and the rest have much lower frequencies.

Table I. Haplotypes Linked to the β^S Gene in Oman*



	Af l III	Hc II	Xmn I	Hd III	Taq I	Hd III	Hc II		Rsa I	Hf I	Fw by DGGE	N.	%
Benin	+	-	-	-	-	-	-	+	-	+	2	57	48.7
Benin-A1	-	-	+	+	+	-	+	+	-	+	2	1	0.85
Benin-A2	+	-	-	+	-	-	-	-	-	+	2	1	0.85
Benin-A3	-	+	+	+	+	-	+	+	-	+	2	1	0.85
Benin-A4	+	-	-	+	nd	-	-	+	-	+	2	1	0.85
Arab India	-	+	+	+	+	-	+	+	+	-	2	30	25.8
AI-A	+	+	-	-	-	-	-	-	+	-	2	1	0.85
Bantu	-	-	-	+	+	-	-	-	+	+	1	24	20.5
Bantu-A	-	-	-	+	+	+	-	+	+	+	1	1	0.85
Total												117	100

*Upper diagram depicts the β^S gene cluster and polymorphic endonuclease sites analysed. In the lower part of the diagram we depict the haplotypes found in Oman linked to the β^S gene and their DGGE frameworks. The final column is the percentage of these haplotypes in the chromosomes studied. 'A1' means atypical 1; and the same nomenclature was used for atypicals derived from the Benin, Arab India and Bantu haplotypes and AI stands for Arab Indian haplotype.

TABLE II. The Average and the Range of HbF and HbA_2 in SS Patients With Different Haplotypes

		HbF (%)		HbA_2 (%)	
Haplotype	No.	Average	Range	Average	Range
Benin/Benin	18	8.3	2.8–14.1	3.9	2.7–4.7
AI/AI	9	18.2	10–24	1.9	1.1–2.9
Bantu/Bantu	4	3.6	2.2–6.4	3.7	3.2–4.2
Benin-A4/Benin-A4	1	4.5		4.1	
Benin/Bantu	7	5.5	1.6–10.5	3.8	3.3–4.7
Benin/AI	6	12.6	5.5–23.9	3.2	2.4–4.5
Bantu/AI	1	10.1		3.2	
Bantu/Benin-A4	1	15		4.1	
Benin/Benin-A1	1	7.4		4	
Benin/Benin-A2	1	6.5		3.8	
Benin/Benin-A3	1			3.2	
AI/AI-A	1	10.6		3.1	
AI/Bantu-A	1	10.3			

Table III. Haplotypes, Genotype and HbF and HbA_2 Level in S/Thalassemia and SC patients

Haplotype	Genotype	n	HbF (%) (range)	Hb A2 (%) (range)
Benin/Thal	S/Thal	5	9.4 (3.7–20.3)	5.7 (2–8)
Bantu/Thal	S/Thal	5	8.3 (5–12.3)	5.9 (4.9–7.2)
AI/Thal	S/Thal	2	8.5 (2.4–14.6)	5.2 (4.6–5.9)
Bantu/HbC	SC	1	6.4	4.5

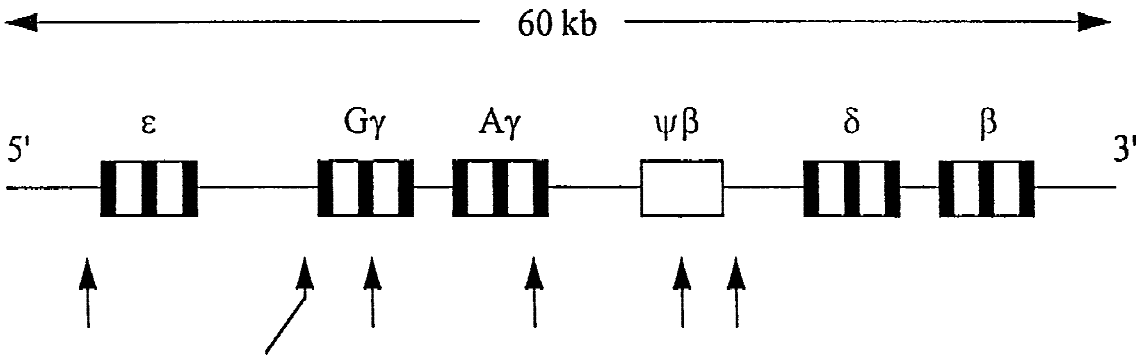
DISCUSSION

To understand the molecular epidemiology of HbS and HbC in Oman is indispensable to review briefly the history of the region, particularly a description of the cultural and population contacts with other peoples [27–31].

Neolithic tools have been found in the eastern portion of the Arabian Peninsula along with Ubaid-type pottery, imported from Mesopotamia, establishing the connection between the Arabian Peninsula and present day Iraq since the end of the last glaciation (10,000 years ago). Little is known about the nomadic people inhabiting the region, except that they cremated the dead and used bifacial neolithic tools. After the third millennium BCE, burials revealed Hafit-type ceramic from Mesopotamia but also autochthonous manufacture of ceramic and decorations.

By 2500–2000 BCE, agricultural settlements (villages) appear dedicated to the domestication of the date-palm (*Phoenix dactylifera*). Development of irrigation and diversification of agriculture using cultivation under the shadow of date-palms followed. Husbandry of sheep, goats, and cattle led to a full-fledged Oasis economy. Wealth and property needed defense, hence fortification emerged.

Table IV. Normal Hemoglobin Linked Haplotypes in Oman



	Hc II	Xmn I	Hd III	Hd III	Hc II		N.	%
ON1	+	-	-	-	-	-	13	56.5
ON2	-	+	+	-	+	+	3	13
ON3	-	-	+	-	-	+	2	8.6
ON4	-	-	-	-	-	+	1	4.4
ON5	-	-	+	-	-	-	1	4.4
ON6	-	-	+	+	-	+	1	4.4
ON7	+	+	+	-	+	+	1	4.4
ON8	-	+	-	-	+	+	1	4.4
Total							23	100

ON = Omani normal haplotype.

Burial sites became elaborate, with the presence of Baluchi/Iranian style pottery and Harappan beads from the Indus valley, establishing the connection of Oman with present day Iran/Pakistan and also the Dilmun empire (vide infra).

A clear cultural divergence occurs, after the third millenium B.C.E., between the northeastern regions of the peninsula [Dilmun empire (Bahrain, Kuwait, Qatar, and the eastern part of Saudi Arabia)] and the Magan and Melukhah empire (in UAE and eastern part of Oman, i.e., inner Oman), with the southern and western regions (Yemen and Saudi Arabia) which established their autochthonous Arabic cultures (Saba, Himyar, Hadhramaut, etc).

A very likely and unwelcome consequence of economical development was the appearance of *malaria*, secondary to the presence of still waters and the concentration of sedentary human population. Malaria had a strong impact on the biology of the population, by selecting for carriers of red cell genetic defects, such as thalassemia, sickle trait, G-6-PD deficiency, known for providing resistance to dying of *Plasmodium falciparum* malaria (reviewed in Ref. 18). These genetic traits are today highly prevalent in this area [11,12]. Today, sickle hemoglobin as well as the spread of malaria is limited predominantly to the northern sections of Oman [13], while the southern part (Dhofar) of Oman has plenty of mosquitos but little malaria or hemoglobinopathies. The answer to this riddle has been proposed to be the indigenous mosquito strain (*Anopheles coustiani*), which does not allow the malaria parasite to reach sporogonia efficiently, interrupting the life cycle of *P. falciparum* [13]. The absence of malaria in southern Al Wusta has been blamed on the scant population of Bedouins and their seminomadic life styles [13].

After the second millennium to 300 BCE, metallurgy appears (weapons, tools) and diversification of the diet occurs, with the incorporation of seafood. The copper trade flourishes in the second millennium B.C., in which Omani copper mined at present day Sohar (Magan em-

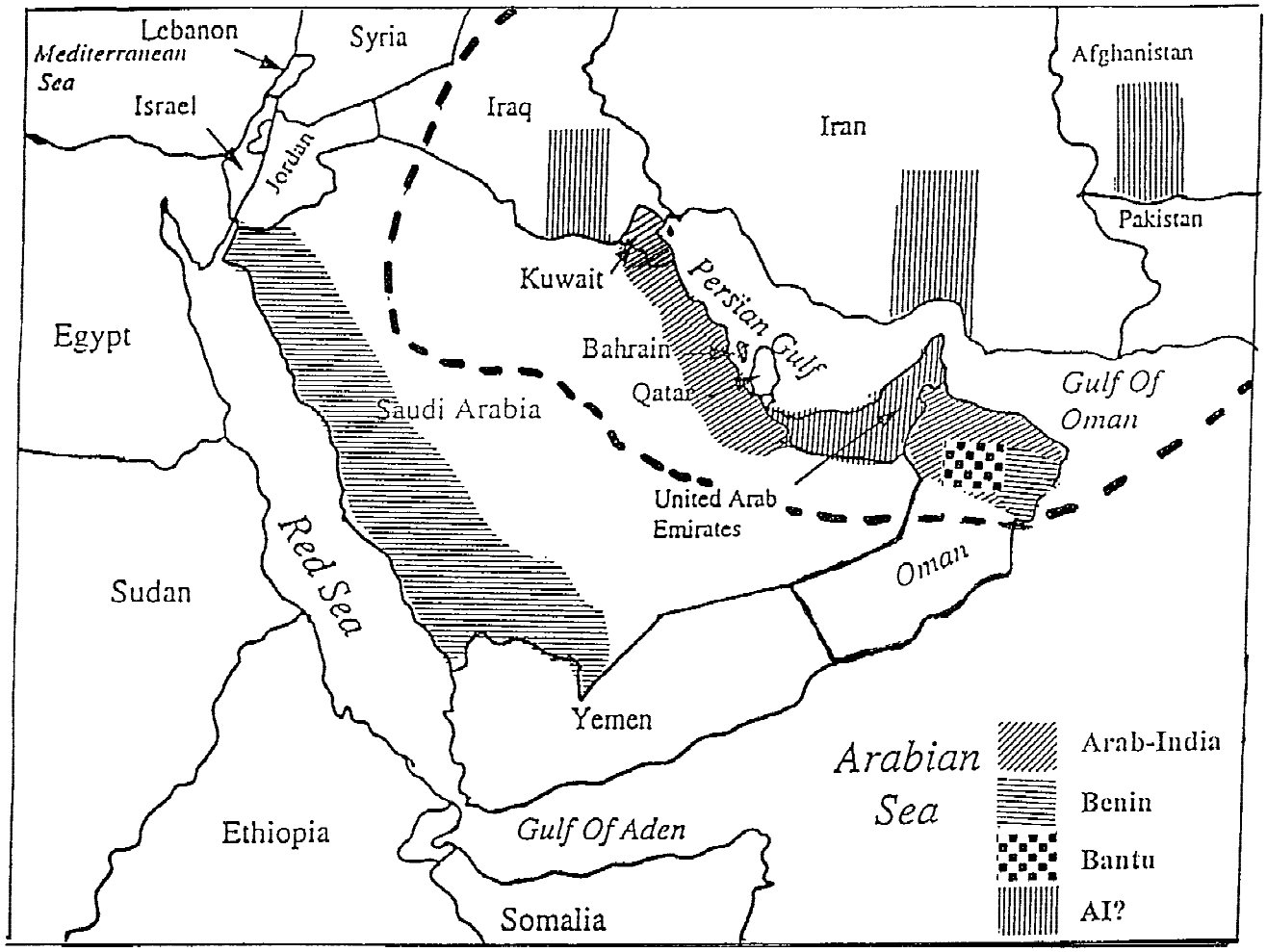


Fig. 1. Map of the Middle East and distribution of haplotypes present in different regions. The dashed line represents the limits of the Sassanian Empire.

pire) moved through the Dilmun culture and was traded in the Mesopotamian city of Ur. Burial remains containing post-Harappan pottery establish continuous contact of Oman with Iraq and the Indus valley. Bronze weapons from Nizwa also point to an Iranian connection.

The first millennium BCE brought the domestication of the camel and the development of the falaj (irrigation canals), a marvel even today, capable of transporting water from the aquifers to the cultivated fields through tunnels. The falaj and bronze hoe resulted in an explosive increase in villages and oases. Domestication of wheat and barley, husbandry of new animals, and iron use flourished. Of course, these developments brought, most likely, an increase in endemic malaria in northern Oman.

The CE finds the eastern Arabian Peninsula heavily influenced by a declining Roman Empire. For Oman it meant the opening of the trade routes to the Orient that began at Palmyra, Syria, through Seleucia in Iraq, to the Characene maritime corridor to Oman, and on to Barigaza in India, creating new contacts between the northeastern oases of the Arabia and the India subcontinent. Sailors from Oman reached India in the first century CE. A decline of the economy followed, with the incorporation of northern Oman to the Archaemenid empire and subsequently domination by the Persian Parthian dynasty.

The period of 200–600 CE was characterized by the great influence of Sassanian monarchs from Persia and religious beliefs included autochthonous paganism, Sassanian Zoroastrianism, and Nestorian Christianity. The latter flourished particularly among the islands between Qatar and Oman. In Figure 1 we include the limits of the Sassanian empire in this period, since it encompasses the region in which the Arab-India haplotype is distributed in the Middle East. We will return to this important point (vide infra). At 200 CE the Omani frankincense commerce, in southern Dhofar, reached its height, connecting the Omanis with northern Yemen and creating considerable wealth until the 7th century.

Around the mid-7th century CE, Oman was among the

earliest to convert to Islam. The Prophet sent emissaries to Sohar and massive conversions followed. Shortly after, Oman came under the rule of the Umayyad dynasty and adopted the Ibadi doctrine, one of the original forms of Islam. The Arabization of Oman began in the early period of the BCE, when Arab tribes moved from Yemen to southern Oman. The next step, the Islamization of Oman increases the Arabization and Arabic became the predominant language.

The appearance of the Portuguese in the 16th century affected little Oman and when expelled in 1650, they left little behind, except for a few forts and the influx of Baluchistanis. The Portuguese confronted and employed Baluchi soldiers in Oman in the 16th and 17th centuries. They were also deployed during the civil wars of the 18th century. This immigration continues until 1958 when Omani's Goiter island, on the Baluchistan coast, was sold to Pakistan.

At the turn of the 19th century Oman became a protectorate of Britain, but not a colony, connecting Oman with India more strongly. The independence from other European powers permitted the Omanis to project their power and commerce southward, and by the middle of 19th century, they controlled Mombasa and the island of Zanzibar in East Africa.

In summary, relevant to genetic epidemiology are the following facts: little is known of the origin of the Omanis, but they became Arabized during the end of the BCE and beginning of the current era, but had strong and active contacts since before the third millennium BCE with Mesopotamia and later on with Persia, the Indus Valley, India, and Yemen. In addition, in mid 19th century, Oman had political and trade contacts with Mombasa and Zanzibar. The influx of Baluchis occurs between the 18th and the 20th centuries.

From the above it is clear that the presence of the Arab-India haplotype linkage to β^S can be explained on the basis of the ancient contacts between Oman and Iraq, Iran and the Indus valley as well as with India. This Indo-European form of sickle cell anemia has been postulated to have originated in the Indus Valley [4], but definitive evidence is not available, and an alternative explanation is its origin among the Mesopotamia and/or Semitic peoples of the Arab Peninsula. Because of the links to India during the British protectorate, it is marginally possible that some of these β^S-linked chromosome to the Arab-India haplotype came with immigrants recruited from the Indian tribals.

The Semitic/Mesopotamia origin of the Arab-India haplotype linked to the β^S gene is unlikely. In areas adjacent to Iraq (Syria, Israel, and Jordan) [32–34], β^S is linked to the Benin haplotype and not the Arab-India haplotype. Moreover, Harappan settlements have not been found around Iraq, nor was this region part of the Sassanian empire. Furthermore, the recent finding of the Arab-India haplotype linked to β^S in Kuwait [10], Bahrain [9], and one SS patient from Qatar with Arab-Indian/Benin haplotype [35], as well as its presence in Oman as shown here, strengthens the idea that this Indo-European form of β^S gene originated in the Indus valley, migrated (gene flow) during the Sassanian Persian empire (224–651 BCE) to the eastern portions of the Arabian Peninsula (including Oman), Iraq, Afghanistan, the Indus Valley, and the Caucasus region, when this region was united under Magian Zoroastrianism. Interestingly, the Saudi Arabia western provinces, where this haplotype does not exist or is very rare (<1) in Western Saudi Arabia [36,37], were outside of the Sassanian empire.

The predominant presence of the Benin haplotype linked to the β^S gene in Oman can be easily explained by the Arabization of the eastern regions of the peninsula at the dawn of the current era by peoples living in the western regions. This period encompassed the Arab slave trade in Africa that involved, at first, mostly regions in which the Benin haplotype was linked to β^S. The growth of agriculture and wealth in this period most probably increased substantially the slave trade from sub-Sahara Africa, explaining the predominance today of the Benin haplotypes. In the western regions of the Arabian Peninsula, as expected, the β^S gene is linked almost exclusively to the Benin haplotype. Finally, the more recent, but close contacts between Oman and Mombasa and Zanzibar, present day Tanzania, give a clear explanation for the presence of the Bantu haplotype linked to β^S in this sample.

The five atypical haplotypes found (Table IV) are interesting. When the 5′ region of each one of them is considered, it becomes apparent the Benin A1 corresponds to the recombination of ON2 with a Benin haplotype. Benin A2 corresponds to a recombination with ON5 and a Benin haplotype. The Benin A3 corresponds to a recombination with ON7 and a Benin haplotype. Bantu A1 haplotype is the result of recombination between ON6 and the Bantu haplotype. The Arab-India A1 haplotype is the product of recombination between ON1 and the Arab-India haplotype.

The frequency distribution of the haplotypes reported here should not be considered definitive since our sample was affected by bias of ascertainment (hospitalized patients), in which the clinical severity differences between the haplotypes could distort the frequencies. A survey of heterozygotes for β^S genes will be needed to obtain the true distribution of these haplotypes among the Omani. The results are, of course, completely valid as to the presence in Oman of the β^S genes linked to the various haplotypes described.

Establishing the haplotypes linked to the β^S gene is important because of the hematological and clinical relationships with individual haplotypes. The Arab-India and the Senegal haplotypes have been associated with the

most benign clinical courses [7,8,38,39,40]. The correlation of HbF level with the linked haplotype in SS patients reveals that homozygosity for the Arab-India haplotype results in the highest HbF. In addition if the homozygotes are divided by gender, the average for HbF of males is 14.5%, while for females it is 23.3%. This situation is reminiscent of the Senegal haplotype, in which its combination with female gender produces the highest level of HbF in sickle cell anemia. [40].

Heterozygotes for the Arab-India haplotype, results in an average of low HbF but with a large range. Nevertheless, no gender effect was found among these genotypes. However, Bakioglu et al. [35] reported five adults from Qatar, Turkey, and South Africa with mild sickle cell anemia who are compound heterozygotes for β^S haplotype (Arab-India/Benin) which have high levels of Hb F (average 22.2%).

The known correlation of sickle cell anemia phenotype with haplotypes predicts that in Oman, patients with sickle cell anemia will be more severe if they carry the Benin haplotype, but especially the Bantu haplotype [41,42]. On the other hand, if they carry the Arab-India haplotype they will have a more benign course particularly if the patient is of the female gender. The high frequency of α-thalassemia will diminish the severity of all patients. Evidence from India demonstrates that α-thalassemia has a profound effect on the phenotype of the disease when interacting with the Arab-India haplotype [43].

In conclusion, this study of HbS in Oman has determined the multicentric origin of the sickle mutation in this population, in which three major haplotypes co-exist (Benin, Arab-India and Bantu) in excellent agreement with the historical record. In addition, the appearance of the Arab-India haplotype in this population, reinforces the hypothesis that the mutation originated in the Harappa culture or in a nearby population, and in addition reveals that the Sassanian empire might have been the vehicle or context by which this Indo-European sickle mutation migrated (gene flow) to the present-day locations.

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